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A tyrosine kinase profile of prostate carcinoma

(oncogene/erbB kinase/signal transduction/human xenograft/reverse transcriptase–PCR)

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ABSTRACT **Tyrosine kinases play central roles in the growth and differentiation of normal and tumor cells. In this study, we have analyzed the general tyrosine kinase expression profile of a prostate carcinoma (PCA) xenograft, CWR22. We describe here an improved reverse transcriptase–PCR approach that permits identification of nearly 40 different kinases in a single screening; several of these kinases are newly cloned kinases and some are novel. According to this, there are 11 receptor kinases, 9 nonreceptor kinases, and at least 7 dual kinases expressed in the xenograft tissue. The receptor kinases include erbB2, erbB3, Ret, platelet-derived growth factor receptor, *sky*, *nyk*, *eph*, *htk*, *sek* (*eph*), *ddr*, and *tkt*. The nonreceptor kinases are *lck*, *yes*, *abl*, *arg*, *jak1*, *tyk2*, and *etk/bmx*. Most of the dual kinases are in the mitogen-activating protein (MAP) kinase-kinase (MKK) family, which includes *MKK3*, *MKK4*, *MEK5*, and a novel one. As a complementary approach, we also analyzed by specific reverse transcriptase–PCR primers the expression profile of *erbB*/epidermal growth factor receptor family receptors in a variety of PCA specimens, cell lines, and benign prostatic hyperplasia. We found that *erbB1*, *-2*, and *-3* are often coexpressed in prostate tissues, but not in *erbB4*. The information established here should provide a base line to study the possible growth and oncogenic signals of PCA.**

Prostate carcinoma (PCA) is the most commonly diagnosed invasive cancer and the second most common cause of cancer death among males in the United States (1, 2). Compared to other more extensively investigated cancers such as breast and colon carcinomas, the present understanding of the oncogenic signals involved in the transformation and progression of PCA lags significantly behind. We recently described (3) a serially transplantable prostate cancer xenograft, designated CWR22, that has a functioning androgen receptor and makes high levels of prostate-specific antigen. Xenografts offer a clonal, pure population of human tumor cells without extensive selections *in vitro*. They are also amenable to genetic manipulations, and could be developed into a useful *in vivo* model to study the mechanisms of oncogenesis. As a first step in understanding the growth signals involved in PCA, we report here our analysis of the *erbB*/epidermal growth factor receptor (EGFR) family of kinases and the general profile of tyrosine kinases in CWR22.

We chose to study tyrosine kinases in PCA because tyrosine kinases are a major class of protooncogenes. While their abundance accounts for less than 10% of all kinases in a cell, nearly all of them are involved in growth signaling and therefore have a disproportionately high chance to be activated as oncogenes. The amplification of *erbB2/neu* correlates well with nodal positive breast carcinomas and serves as a paradigm of using an activated oncogene as a tumor marker and therapeutic target (4). Recent evidence suggests that tyrosine kinases can physically interact with one another [e.g., *src*/platelet-derived growth factor receptor (PDGFR) (5), *src*/*erbB1* (6), *erbB1/erbB2* (7), etc.] and form an interconnecting circuitry for signal transduction. As a consequence, we believe that a combined activation profile of tyrosine kinases is needed to fully appreciate the oncogenic pathways, and such a profile may serve as a better oncogenic or tumor marker than any single kinase.

Among the receptor tyrosine kinases, the *erbB*/EGFR family is the most frequently implicated in human cancers (8). The coexpression of the *erbB*/EGFR and its ligands (transforming growth factor α, EGF, or amphiregulin) is a common occurrence in many cancers of epithelial origin. All three widely used PCA cell lines, PC3, DU145, and LN CaP, express *erbB1* and manufacture transforming growth factor α or EGF, establishing autocrine loops for growth (9–12). Overexpression of *erbB2/neu* has also been recently reported in PCAs (13–16). Considerably less is known about the expression of *erbB3* and *erbB4* as well as their ligand, heregulin (also known as neu differentiation factor; ref. 17) in PCAs. We recently reported that CWR22 expresses *erbB1*, *-2*, and *-3* but not *-4*, and that it expresses a significant level of HRG, but not transforming growth factor α (3). Here we have extended this analysis by using specific reverse transcriptase (RT)–PCR primers to examine the profile of *erbB* family members in PCA xenografts, cell lines, and (benign prostate hyperplasia (BPH). Our general conclusion is that the majority of PCAs coexpress *erbB1*, *-2*, and *-3*, but rarely *-4*.

To extend the analysis to other tyrosine kinases, known or unknown, we sought to develop an approach that can efficiently provide a general tyrosine kinase profile in PCA. RT–PCR using degenerate primers has been used effectively to identify novel kinases (see, for examples, refs. 18 and 19); but in most instances, a small number of tyrosine kinases were uncovered. We have made several modifications of this approach such that in a single screening, up to 40 different kinases can be identified. Here, we report a detailed tyrosine kinase profile of a PCA.

MATERIALS AND METHODS

Cell Lines and Tumor Specimen. DU145, LNCaP and PC3 PCA cell lines were purchased from the American Type Culture Collection. The propagation of xenografts CWR22, -31, -21, and -91 was as described (3). All other tumor samples were obtained through the tissue procurement network under the direction of one of the authors (T.P.). All PCA samples used in this study contain at least 80% of tumor tissues as verified by histopathological examination.

RT–PCR Profile of *erbB* Family Receptors. Briefly, the RNA was isolated by a guanidine isothiocyanate method (20). Fifty micrograms of total cellular RNA was used for reverse

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Abbreviations: PCA, prostate carcinoma, EGFR, epidermal growth factor receptor; RT, reverse transcriptase; BPH, benign prostatic hyperplasia; PDGFR, platelet-derived growth factor receptor; MAP, mitogen-activating protein; MKK, MAP kinase kinase; PRPP, phosphoribosyl pyrophosphate synthetase.

transcription, with oligo $(dT)_{12-18}$ as primers. The reaction conditions were as described (3). PCR was carried out in 10 mM Tris·HCl (pH 9.3), 50 mM KCl, 2.5 mM $MgCl_2$, 0.1% Triton X-100, and 200 μM concentrations of each deoxynucleotide triphosphate for 30 cycles, with a cycle profile of 50 s at 95°C, 2 min at 60°C, and 2 min at 72°C, followed by a 7-min extension at 72°C. PCR products were analyzed by 4% PAGE. The primers used are as described (3). The predicted molecular sizes of the PCR products are, respectively, 726 bp (*erbB1*), 821 bp (*erbB2*), 1129 (*erbB3*), and 1013 bp (*erbB4*).

RT–PCR Profiling of Tyrosine Kinases. The RNA isolation and cDNA synthesis are as described in the previous section except 5 μg of poly(A)$^+$ RNA was used in the initial cDNA synthesis and 20 ng of cDNA was used for each PCR. The PCR primers are derived from the conserved motifs DFG and DVW with an asymmetric *Bst*EII site (underlined) contained at the 5′ end of each primer. For the 5′ primer, the sequences *Bst*EII (GGTCACC)-K [V/I] [SCG] D F G and *Bst*EII (GGTCACC)-K [V/I] [AST] D F G were included, and for the 3′ primer, D V W [S/A] [F/Y] G-*Bst*EII (CCATTGG) was used. The PCRs were conducted at 42°C annealing temperature for 5 cycles and then at 55°C for 25 cycles. A fraction of the initial PCR products was further divided into different pools and digested with restriction endonucleases *Mbo*I, *Rsa*I, *Hae*III, or *Alu*I. These pools were reamplified with the original set of primers for 15 cycles with 55°C annealing temperature. The 165- to 171-bp band from the undigested pool as well as from those digested pools were purified from the agarose gels. The purified fragments from the different pools were digested with *Bst*EII and cloned into mp18BstEII, a M13 replicative form DNA modified to carry the asymmetric tandem *Bst*EII cloning sites. mp18BstEII was constructed by site-directed mutagenesis of the multiple cloning site of M13 mp18 replicative form to replace the *Bam*HI-*Xba*I-*Sal*I sequence by the tandem asymmetric *Bst*EII sites (GGTCACCTAGAGGTAACC). The resulting recombinants were used to electro-transform *Escherichia coli* XL-Blue (Stratagene) and scored as colorless plaques on 5-bromo-4-chloro-3-indolyl β-D-galactoside (X-Gal) plates. These plaques were transferred to individual 5-ml cultures of *E. coli* XL-Blue. After 8–10 h, the single-stranded phage DNA were prepared by the polyethylene glycol (PEG) precipitation/phenol extraction/ethanol precipitation method. DNA from each culture was suspended and used as a template for chain-terminator sequencing in the presence of dideoxy T. These "T-tracks" were compared and used to identify the different kinase species. Clones representing each species were sequenced by using all four dideoxy reactions (21). The sequences were compared with the Entrez databank.

RT–PCR Expression Analyses of Selected Kinases. Total RNA samples (50 μg) from various PCA xenografts and cell lines were used in reverse transcription reactions. The conditions were the same as described in the previous section except a degenerate primer W S/A FL/YC G was used instead of oligo dT. This primer overlaps with the DVW S/A F/Y G primer used in the profiling reaction and significantly enriches the kinase-related transcripts, thus enhancing the sensitivity. The resulting cDNAs (from approximately one tenth of the reverse transcription reaction) were then subject to PCRs by using gene-specific primers. The PCR was conducted in 50 μl containing 200 μM each dNTP, 1.8 mM $MgCl_2$, and 200 nM each of the specific primer for 30 cycles at 60°C annealing temperature for 1 min, 72°C for 1 min, and 94°C for 45 min. The primers sets for the three kinases studied are as follows: Arg (sense, GAGAGGTTTACGTTGGCGTCTG; antisense, TTTGGCTCCAGCATGAGCAG), Ret (sense, GGCAGCCAGAAACATCCT; antisense, ACTTTGCGTGGTGTAGAT), and MAK (sense, GCAAGAGAATTAAGGTCACAGCC; antisense, GGGAGAACTATAAACTGAAGATCTC). The expected sizes of PCR products, respectively, are 440 bp for Arg, 172 bp for Ret, and 102 bp for MAK. The primers for internal RT and PCR control, phosphoribosyl pyrophosphate synthetase (PRPP) (22), are sense, TCGCTTAGTGGAGTGCTTAGG and antisense, GTCATTGATTTCGCCACAAC, with an expected RT–PCR product of 239 bp.

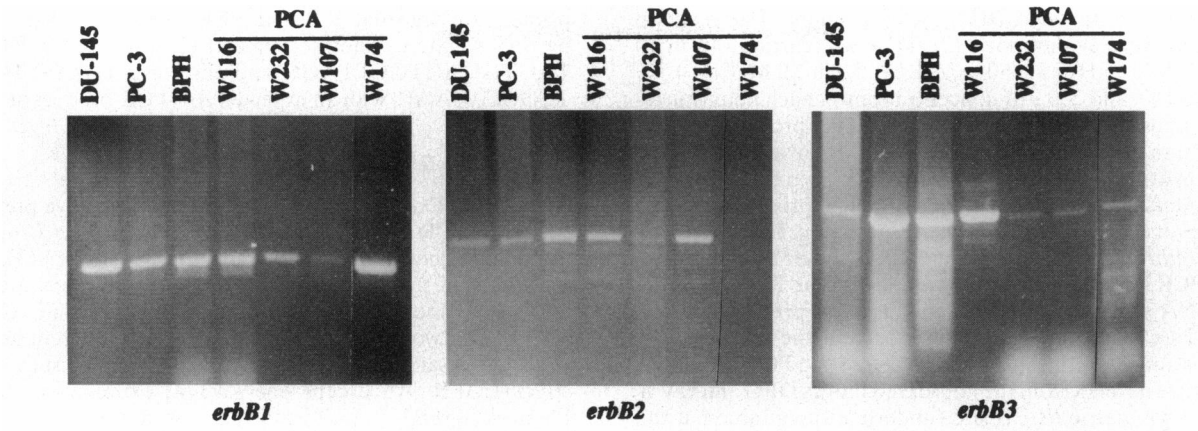
RESULTS AND DISCUSSION

The *erbB*/EGFR Family Profile in PCA. We have previously designed PCR primers that are present in the C-terminal regions unique to individual *erbB* family members (3). Using these sets of primers, we have analyzed two prostatic carcinoma cell lines, two xenografts derived from PCAs, eight PCAs, and two BPHs. The data were summarized, and representative samples are shown in Fig. 1. All tissues express *erbB1*/EGFR. All except one (W174) expresses *erbB2/neu*. Likewise, *erbB3* is uniformly expressed except one tumor (W006). *erbB4*, on the other hand, is not expressed in any of the prostate tissues. Thus, there is a general consistency as to the presence of EGFR family members. Our study calls attention to the *erbB3* receptor as well. The presence of this receptor has profound effect on the signaling by *erbB1* and *-2*. *erbB3* receptor may lack an intrinsic kinase activity (23), but pairs with *erbB1* (24) and *erbB2* (25) to diversify the signals. It is noteworthy that CWR22 releases HRG, the ligand for *erbB3* (3) and forms an autocrine loop.

The Tyrosine Kinase Profile of PCA. Having established the profile of *erbB* family receptor, we wished to describe the overall pattern of tyrosine kinases in PCAs. As discussed before, RT–PCR with degenerate primers has previously been used to identify novel tyrosine kinase clones. Its utility to generate a representative expression profile of tyrosine kinases has been somewhat limited. We have modified the protocol so that a large number of kinases can be obtained in a single screen. Specifically, we used a degenerate-primer set that encode invariable sequences of the DFG and DVW regions (26), coupled with an improved vector system.

The cDNA was synthesized by oligo-dT priming. PCRs were carried out on the cDNA by using the degenerate DFG and DVW primer pair as described in *Materials and Methods*. A divergent *Bst*EII site was attached to the 5′ end of each primer to facilitate directional cloning. This exploits the unique property of *Bst*EII, which recognizes sequences with wobble nucleotides in the middle—i.e., CCACTGG and CCATTGG. CCACTGG was attached to the 5′-DFG primer and CCATTGG to the 3′-DVW primer. With this set of primers, we anticipated and obtained a relatively homogeneous PCR product of 161–170 bp (Fig. 2). This band was purified, digested with *Bst*EII, and cloned into mpBstEII, a M13 mp derivative (27) modified to accept the divergent *Bst*EII ends. Fractions of the PCR product were digested with various restriction endonucleases with four-base recognition sites and reamplified before digestion with *Bst*EII. This was to avoid possible dominance of certain overexpressed clones. In a single experiment, we obtained about 2000 clones and analyzed 600 of them. Individual clones of M13 phages were isolated, and the single-stranded DNA used as templates for chain-termination sequencing (21) in the presence of dideoxy T. These T-tracks were compared to identify the different kinase species. Based on this analysis, >90% of the clones are protein kinases and a total of 43 different species were identified. Clones representing each species were then sequenced by using all four dideoxy nucleotides. Multiple clones were sequenced to minimize the variations due to errors introduced in PCRs. The data, which are summarized in Table 1, show that among the 43 kinases identified, ≈30 are human tyrosine kinases. The rest were of murine origin derived from the contaminating mouse stromal and hemopoietic cells in the xenograft tissue.

Among the human tyrosine kinases, three types are identified: the receptor tyrosine kinase, the nonreceptor tyrosine kinases, and the dual/other kinases. The upper column in



	Cell line		xenograft		BPH		PCA							
	DU145	PC3	CW22	CW31	W029	W043	W006	W107	W116	W137	W171	W174	W214	W232
erbB1	+	+	+	+	+	+	+	+	+	+	+	+	+	+
erbB2	+	+	+	+	+	+	+	+	+	+	+	-	+	+
erbB3	+	+	+	+	+	+	-	+	+	+	+	+	+	+
erbB4	-	-	-	-	-	-	-	-	-	-	-	-	-	-

FIG. 1. The *erbB*/EGFR family receptor expression profile in BPH and PCA. Total cellular RNAs from prostatic carcinoma specimen (PCA), PCA cell lines, PCA xenografts, and benign hyperplasia (BPH) were used as templates for RT–PCR. The conditions and the specific primers for *erbB1*, *-2*, *-3*, and *-4* are as described (3). Based on histological examination, all PCA samples analyzed here contain at least 90% tumor cells. W029 is 100% BPH and W043 consists of 30% BPH and 70% prostate intraepithelial neoplasia. The xenografts are about 80% human tumor cells and 20% murine stromal cells. Samples with detectable RT–PCR bands are labeled as +; and those with undetectable bands of the right size are labeled as −.

Table 1 shows the receptor tyrosine kinases. There are 11 of them that fall into five subfamilies. We observed *erbB2* and *erbB3* expression, consistent with the results using specific primers (*erbB1* happens to have a *Bst*EII site within the amplified kinase region and thus was not in the 170-bp fragment pool). *ret*, a gene implicated in multiple endocrine neoplasia (28) and PDGFR-β (29), is also expressed. There are three members (*eph*, *htk*, *sek*) of the *eph* family (30–32) and two members (*sky* and *nyk*/*mer*) of the *ufo*/*axl* family receptors (33–35). *ddr* and *tkt* are two recently identified kinases with a discoidin I motif in the extracellular domain (36, 37). *sky*, *nyk*, *ddr*, and *tkt* are kinases likely to be involved in signaling through cell–cell contact. Among the 11 receptor kinases, at least four of them (*erbB2*, *ret*, PDGFR, and *nyk*) are either themselves or their homologs in other species have been shown to be potentially oncogenic.

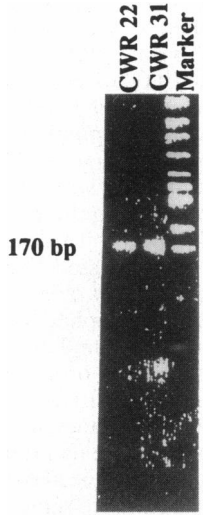


FIG. 2. RT–PCR products of CWR22 and -31 xenografts using degenerate primers. Poly(A)$^+$ RNAs from xenograft CWR22 and -31 were used as templates in RT–PCR with DFG and DVW degenerate primers. The reaction conditions are described. The PCR products, together with *Hae*III-digested λ DNA molecular size marker, were analyzed in a 4% agarose gel and visualized by ethidium-bromide staining and UV illumination. The 165- to 171-bp fragment of CWR22 was subsequently used for cloning and identification of the expressed kinases.

The middle panel in Table 1 shows the seven nonreceptor tyrosine kinases expressed in CWR22. *yes* (38) and *lck* (39) are in the *src* family and *abl* (40) and *arg* (41) are in the *abl* family. *tyk2* (42) and *jak1* (43), the two kinases involved in the signaling of a large number of cytokines (interferon, interleukin 3, interleukin 6, etc.), are also expressed in this xenograft. *etk*/*bmx* (44) is a newly identified kinase expressed in breast tumors, which belongs to the *btk* family and is encoded by a X-linked gene. Among these seven kinases, *yes*, *lck*, and *abl* are known protooncogenes.

The remaining clones are grouped as dual and other kinases. Many of them are novel; thus, there is no sufficient basis to classify them as dual kinases or strictly serine/threonine kinases. The dual kinases have both tyrosine and serine/threonine kinase activities, and the most well-defined group is the mitogen-activating protein (MAP) kinase-kinase (MKK) family (45) These kinases serve as the integrators of signals coming from various receptor tyrosine kinases, nonreceptor tyrosine kinases, and G protein-linked receptors. Their immediate substrates are MAPK/ERK, SAPK/JNK, and p38K/HOGK, a group of serine/threonine kinases that have the potential to interact directly with transcriptional factors and whose activations rely on phosphorylations at both tyrosine and serine/threonine residues (46). We identified at least four different dual kinases in PCA that belong to the MKK family. Since our initial identification, three of them have been identified as SEK (or JNKK, MKK4) (47–49), P38K (or MKK3) (47), and MEK5 (50), with the remaining kinase (MEK5-like) yet to be clone-isolated. Clk3 (51) belongs to a different family of dual kinases (i.e., *clk*/*sty* family) that are likely to exert their effects in the nucleus. The rest of the kinases are related to serine/threonine kinases although some of them [MAK (52), EMK (53), and homolog to C.el.C058H

Table 1. Tyrosine kinases profile in CWR22 PCA

Sequence	Identity
RECEPTOR TK	
DFGLARLLDI--DETEYHA-DGGKVPVKWMALESILRRRFT----HQSDVW	erbB2/neu
DFGVADLLPPDDKQLLYS---EAKTPIKWMALESIHFGKYT----HQSDVW	erbB3
DFGLSRDVY---EEDSYVKRSQGRIPVKWMAIESLFHHIYT----TQSDVW	Ret
DFGLARDIM---RDSNYISKGSTFLPLKWMAPESIFNSLYT----TLSDVW	PDGF-B
DFGLTRLL--DDFDGTYETQ-GGKIPIRWTAPEAIAHRIFT----TASDVW	Eph
DFGLSRFLEENSSDPTYTSSLGGKIPIRWTAPEAIAFRKFT----SASDVW	Btk
DFGMSRVL-EDDPEAAYTTR-GVKIPIRWTAPEAIAYRKFT----SASDVW	Sek(eph)
DFGLSRKIY---SGDYYRQGCRSKLPVKWLALESLADNLYT----VQSDVW	Sky/Tyro3
DFGLSKKIY---SGDYYRQGRIAKMPVKWIAIESILADRVYT----SKSDVW	Nyk/Mer
DFGMSANL---YAGDYYRVQGRAVLPIRWMAWECILMGEFT----TASDVW	Ddr
DFGMSRNL---YSGDYYRIQGRAVLPIRWMSWESILLGKFT----TASDVW	Tkt
NON-RECEPTOR TK	
DFGLARLI----EDNEYTAREGAKFPIKWTAPEAINYGTFT----IKSDVW	Lck
DFGLARLI----EDNEYTARQGAKFPIKWTAPEAALYGRFT----IKPDVW	Yes
DFGMSRLM----TGDTYTAHAGAKFPMKWTAPESLAYNKFS----IKSDVW	Abl
DFGLSRLM----TGDTYTAHAGAKFPIKWTAPESLAYNTFS----IKSDVW	Arg
DFGLAKAV--PEGHEYYRVREDGDSPVFWYAPECLKEYKFY----YASDVW	Tyk2
DFGLTKAI--ETDKEYYTVKDDRDSPVFWYAPECLMQSKFY----IAYDVW	Jak1
DFGMTRYV----LDDQYVSSVGTKFPVKWSAPEVFHYFKYS----SKSDVW	Etk/Bmx
DUAL TK & OTHERS	
DFGISGYL------VDSVAKTMDAGYKPYMAPERINPELNQKGYNVKSDVW	Mkk3/p38K
DFGISGQL------VDSIAKTRDAGCRPYMAPERIDPSASRQGYDVRSDVW	Mkk4/sek
DFGVSTQL------VNSIAKTY-VGTNAYMAPERISGEQYG----IHSDVW	MEK5
DFGVQAQL------VNSIAKTY-VGTNAYMAPERISGEQYG----IHSDVW	*MEK5-like***
DFGSATFD--------HEHHTTIVATRHYRPPEVILELGWA----QPCDVW	CLK3
DFGLAREL------RSQPPYTDYVSTRWYRAPEVLLRSSVY---SSPIDVW	*MAK***
DFGVSNEF-----KGSDALLSNTVGTPAFMAPESLSETRKI-FSGKALDVW	*CO5H8-like***
DFGFSNEF------TFGNKLDTFCGSPPYAAPELFQGKKYD---GPEVDVW	*Emk**
DFGLSNMM------SDGEFLRTSCGSPNYAAPEVISGRLYA---GPEGDVW	AMPK
DFGLAIEV-----QGDQQAWFGFAGTPGYLSPEVLRKEAYG----KPVDVW	*CamKIIβ**
Murine TK	
DFGMSRVL-EDDPEAAYTTR-GGKIPIRWTAPEAIAYRKFT----SASDVW	mu-sek(eph)
DFGMTRFV----LDDQYTSSTGTRFPVKWASPEVFSFSRYS----SKSDVW	mu-tsk
DFGMTRDI---YETDYYRKGGKGLLPVRWMSPESHKDGVFT----THSDVW	mu-FD16(IRR)
DFGLARII----EDNEYTAREGAKFPIKWTAPEAINFGSFT----IKSDVW	mu-bmk/hck
DFGLARLL--DIDETEYHA-DGGKVPIKWMALESILRRRFT----HQSDVW	mu-erbB2
DFGLARDIM---NDSNYVVKGNARLPVKWMAPESIFDCVYT----VQSDVW	mu-fms
DFGLSRG------EEVYVKKTMGRLPVRWMAIESLNYSVYT----TKSDVW	mu-tie
DFGISGYL------VDSVAKTMDAGCKPYMAPERINPELNQKGHNVKSDVW	mu-Mkk3
DFGFTNEF------TFGNRLDTFCGSPPYAAPELFQGKKYD---GPEVDVW	mu-Emk
DFGMCKEN-----IWDGVTTKTFCGTPDYIAPEIIAYQPYG----KSVDVW	mu-PKCβ
DFGVQAQL------VNSIAKTY-VGTNAYMAPERISGEQYG----IHSDVW	*mek5-like***
DFGVSNEF-----KGSDALLSNTVGTPAFMAPESLSETRKI-FSGKALDVW	*CO5H8-like***

The ** indicates novel clones whose sequences do not match any human clones in the Entrez databank. The * indicates clones of which only partial cDNA sequences are available from the databank; the known kinases that show the highest homology to these clones are denoted in the Identity column. The assignment of some of the novel clones (e.g., human and murine MEK5-like kinase) to the human and mouse categories are presently arbitrary; these clones have identical amino acid sequences, but with multiple differences at the nucleotide level that cannot be accounted for by PCR errors.

(54)] have not had complete human cDNA clones reported. Whether they have any tyrosine kinase activity awaits functional analysis.

Due to the high sensitivity of this approach, we have also picked up kinases from the contaminating mouse tissues, estimated to be 10 -20% of the xenograft populations. A total of 12 kinases were identified. Not surprisingly, many of these kinases such as *fms*, *tsk*, and *tie* are of hemopoietic and endothelial origin, presumably due to vascularization within the xenografts.

The Expressions of the Profiled-Kinases in PCAs. To determine whether the above profile is representative of PCAs in general, we used a semi-quantitative RT–PCR to study the expressions of selected kinases in other PCA cell lines and xenografts. We chose Arg, Ret, and MAK kinases for these studies as they are novel and have never been investigated in PCA samples. Gene-specific primers were used to obtain individual RT–PCR products and a housekeeping gene PRPP (22) was used as a quantitation control. We selected this gene because of its ubiquitous tissue distribution and that it fortuitously shares with the kinase domain a common sequence motif (see *Materials and Methods*), which was used as a primer instead of oligo-dT in the RT reaction. In such a way, PRPP also serves as a proper control of reverse transcription, and its expression is uniform in all PCA samples examined (Fig. 3). All three kinase genes are expressed in not only the CWR22 xenograft where they were initially identified, but also in an androgen-insensitive variant of CWR22 (lane CWR22R), in three other xenografts (lanes CWR31, -21, and -91), as well as

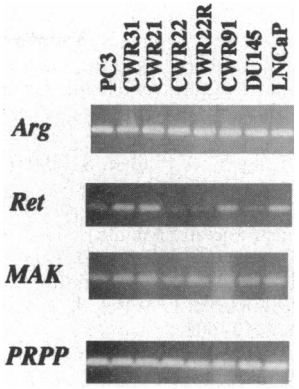


FIG. 3. Expression analysis of selected kinases in PCA xenografts and cell lines. RT–PCR was used to study the general expression pattern of three kinases Arg, Ret, and MAK identified in Table 1. PRPP, a housekeeping gene, was used as a control for both RT and PCRs. Identical aliquots of the reactions were loaded in all lanes. The detailed procedures, and the RT and PCR primers used, are described in *Materials and Methods*. The final products were analyzed in a 4% polyacrylamide gel and visualized by ethidium bromide staining. The PCA cell lines and xenografts used in these analysis are indicated. CWR22R is a relapsed xenograft derived from CWR22 in a castrated animal.

in the three established PCA cell lines (lanes PC3, DU145, and LNCaP). Although the level of Ret expression varies among different samples in a reproducible way, they are detectable in all samples. This finding suggests at least for some of the profiled kinases, the expression is a general property of PCA, not peculiar to CWR22.

A New Approach to Profile Tyrosine Kinases. This paper describes a modified RT–PCR approach to examine the tyrosine kinase expression in a given cancer cell. We present data for a single screen of 600 clones to illustrate the effectiveness and the fidelity of this approach. This screen is by no means exhaustive, and if one delves deeper, more kinases will likely be obtained. Out of the 48 different kinases obtained in this screen, at least 35 are of human origin. Since the CWR22 xenograft is derived from a single clone of human prostate tumor, all the human kinases are of PCA origin. The number of different kinases obtained in our screen is much higher than that reported in previous studies using similar degenerate primer approaches. If one takes 2000 as the total number of kinases encoded by human genome (55), 200 of them would be tyrosine kinases, or 10% of all kinases. It is then reasonable to assume that in a given cell type, the total number of tyrosine kinases expressed would be about 50 or so. The kinase profile obtained here may account for 50% or more of the kinases expressed in CWR22. We attribute our relative efficiency to the selection of primers close to the 3′ end of the kinase domain and flanking a short, but fixed, number of nucleotides (≈120 bp without primers), making this approach less sensitive to mRNA degradation. The relatively homogeneous size distribution makes the purification of a kinase band possible, if nonspecific priming become a serious problem in certain tissue types. The 170-bp overall size can be displayed in a single sequencing gel, making the comparison of different clones easier. The DFG motif is present nearly in all kinases, and DVW is characteristic of tyrosine kinases, although some dual kinases and serine/threonine kinases also contain the latter motif. The above consideratons make DFG and DVW an attractive primer set to profile kinases.

In summary, we report here an efficient approach to describe the expressed tyrosine kinases in a cancer cell and a tyrosine kinases profile for a human prostate cancer xenograft. The information obtained should provide a baseline to study the possible oncogenic pathways involved in PCA development.

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